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Dear Sir or Madam:

Enclosed is a copy of a Health and Safety Study, submitted on behalf of all members of the International Isocyanate Institute, Inc. (BASF Corporation, Dow Chemical Company, ICI Americas, Inc., Miles, Inc., and Olin Corporation). We are filing the final report to satisfy the reporting requirements of 40 CFR 716.

Information required as follows:

Chemical Substances:	Benzene diisocyanatomethyl- (unspecified isomer)
CAS No.:	1321-38-6
Study Title:	Toluene Di-isocyanate: An Evaluation in the Mouse Micronucleus Test
III No.:	E-AB-65
Submitting Official:	R. K. Rigger
Title:	Managing Director
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Very truly yours,

R. K. Rigger
Managing Director

RKR/sha
Enclosure

REPORT

Toluene Di-isocyanate: an Evaluation in the Mouse Micronucleus Test

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III Project E-AB-65

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Date of III issue 10 December 1992

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Summary

Conclusion from report: TDI, under the conditions of test, is not clastogenic in the mouse micronucleus test.

 10995

ICI CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

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TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

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TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

by

J M Mackay

Approved for Issue: J E Doe
Project Manager

Date of Issue 13 OCT 1992

TOLUENE DI-ISOCYNATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

STATEMENT OF GLP COMPLIANCE

To the best of our knowledge and belief, the investigations described in this report were conducted in accordance with the following Good Laboratory Practice standards, except that

(i) there is no documentation to indicate that the test substance characterisation was performed in a GLP-accredited Laboratory

(ii) certified purity and stability of the control substances are not available.

These deviations are considered not to affect the integrity of the study or the validity of the conclusions drawn.

1. United States Environmental Protection Agency (Title 40 Code of Federal Regulations Part 160 - Federal Insecticide, Fungicide and Rodenticide Act)

2. United States Environmental Protection Agency (Title 40 Code of Federal Regulations Part 792 - Toxic Substances Control Act)

3. United Kingdom Department of Health (Annex to the United Kingdom Compliance Programme, 1989): Compatible with OECD 1982 (Good Laboratory Practice in the Testing of Chemicals - Final Report of the OECD Expert Group on Good Laboratory Practice, ISBN 9264 12367 9)

4. Japanese Ministry of Agriculture, Forestries and Fisheries (59 Nohsan No.3850, August 10 1984)

J M Mackay ..... 9 October 1992.
Study Director

G J A Oliver ..... 12 October 92
Regulatory Toxicology Manager
ICI Central Toxicology Laboratory

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

QUALITY ASSURANCE STATEMENT

In accordance with ICI policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Date of QA Report
18 Jan 89	18 Jan 89
7 Feb 89	8 Feb 89
10 Feb 89	13 Feb 89
5 Oct 89	5 Oct 89
6 Sep 91	2 Oct 91
15 Sep 92	16 Sep 92
8 Oct 92	9 Oct 92

Facilities and process based procedures associated with this study were inspected in accordance with QA Standard Operating Procedures.

So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, SM0353.

G P Fuller (Unit Head, CTL Quality Assurance Unit) ....G P Fuller.... ....9 Oct 92...

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

I, the undersigned declare that this report constitutes a true record of the actions undertaken and the results obtained in the above study.

J M Mackay (Study Director) ..... 29 September 1992.

Mrs C A Howard was Study Director from the commencement of the study until 29 August 1989, when she left CTL on maternity leave. From that date Dr J M Mackay assumed the responsibilities of Study Director.

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TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

The following contributed to this report in the capacities indicated:

D Woodcock	(Study Investigator and Home Office Licensee)
K Jones	(Cytogenetic Analyst)
N H James	(Cytogenetic Analyst)
N Cryer	(Analytical Chemistry)
M Abdy	(Analytical Chemistry)
M Greenwood	(Statistician)
P Hext	(Inhalation Toxicologist)

Reviewed by:
B M Elliott (Head, Regulatory Genetic Toxicology) 25.9.92

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

CONTENTS

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

CONTENTS - continued

CONTENTS - continued

CONTENTS - continued

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

SUMMARY

Toluene di-isocyanate (TDI) has been evaluated for its ability to induce micronucleated polychromatic erythrocytes in the bone marrow of C57BL/6JfCD-1/Alpk mice. Groups of 5 male mice were exposed to TDI for a 6 hour period by the inhalation route at target concentrations of 11.8 and 18.9ppm. Groups of 5 female mice were similarly exposed to TDI at target concentrations of 7.5 and 11.9ppm. In both cases these concentrations were selected to represent 50 and 30% respectively of a median lethal concentration (MLC) estimated in that sex over a four day observation period. Due to an error in the original calculation of the MLC values the target concentrations used actually represented approximately 62 and 99% of the MLC in males and 53 and 84% of the MLC in females. Bone marrow samples were taken 24 hours after the end of the exposure period for the lower concentrations and 24, 48 and 72 hours after the end of the exposure period for the higher concentrations.

Small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the air control values, were observed in females 24 hours after being exposed at the target concentrations of 7.5ppm and 24 and 48 hours after being exposed at the target concentration of 11.9ppm. These increases were small and not concentration-related. Extended analysis of a further 2000 polychromatic erythrocytes from these animals and the female air control animals at the 24 and 48 hour time points was conducted. No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes were observed in these extended counts. However, when the original and extended analyses were combined prior to statistical analysis small but statistically significant increases were observed in females 24 hours after being exposed at both target concentrations.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

SUMMARY - continued

Small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the air control values, were observed in males 24 hours after being exposed at the target concentrations of 11.8 and 18.9ppm but there was no clear concentration-response relationship. Extended analysis of a further 2000 polychromatic erythrocytes from these animals and the male air control animals at the 24 hour time point was conducted. A small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes was observed only at the lower target concentration (11.8ppm) in these extended counts and when the original and extended analyses were pooled prior to statistical analysis.

In order to further investigate the increases observed in both males and females exposed to TDI and the lack of concentration-response relationships observed a second assay was conducted. Groups of 5 male mice were exposed to TDI for a 6 hour period by the inhalation route at target concentrations of 5.9, 11.8 and 18.9ppm and groups of 5 female mice were similarly exposed to TDI at target concentrations of 3.7, 7.5 and 11.9ppm. In both cases these concentrations were selected to represent the concentrations used in the first study with an additional lower concentration to investigate any concentration-response relationships. Due to the error in the original calculation of the MLC values, the target concentrations used actually represented approximately 31, 62 and 99% of the MLC in males and 26, 53 and 84% of the MLC in females. Bone marrow samples were taken 24 hours after the end of the exposure period for all concentrations.

In this second study high levels of lethality were observed at the 11.8ppm (62% MLC) concentration in males and the 11.9ppm (84% MLC) concentration in females and therefore the slides from the males exposed at the 5.9ppm

SUMMARY - continued

target concentration and the females exposed at the 3.7 and 7.5ppm target concentrations only were analysed. The maximum concentration in each case is considered to represent a maximum tolerated concentration (MTC) in this second study.

No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, compared to the air control values, were observed in the males exposed to TDI.

Small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the air control values, were observed in females 24 hours after being exposed at both the 3.7 and 7.5ppm target concentrations. These increases were small and the values fell within the range of female air control values reported in this study. It is therefore considered that the increases observed in this second study are due to a low control value rather than to any effect of TDI. The increases are therefore considered not to be biologically significant.

In summary, although increases in the incidence of micronucleated polychromatic erythrocytes were observed in both males and females exposed to TDI these increases were small, not concentration-related and were not reproducible at concentrations limited by lethality in a repeat study. It is therefore considered that the increases observed are of no biological significance and do not indicate any clastogenic activity of TDI in the mouse bone marrow micronucleus assay.

Consideration of the percentage of polychromatic erythrocytes showed statistically significant decreases, compared to the air control values, in both males and females in the first study and in females in the second

SUMMARY - continued

study. These decreases may indicate that TDI or a metabolite has induced a cytotoxic response in the bone marrow resulting in a depression of cell proliferation.

The test system positive control, vinyl chloride, induced statistically significant and biologically meaningful increases in micronucleated polychromatic erythrocytes, compared to the air control values, in both studies thus demonstrating the sensitivity of the test system to a known clastogen.

It is therefore concluded that TDI, under the conditions of test, is not clastogenic in the mouse micronucleus test.

1. INTRODUCTION

Toluene di-isocyanate (TDI) was tested for its ability to induce clastogenic effects using the mouse bone marrow micronucleus test.

The micronucleus test is capable of detecting the clastogenic effect of a chemical. After chromosomal damage has been induced by a test compound or its metabolites, acentric fragments of chromosomal material lag behind at anaphase. At telophase a large proportion of these fragments are not included in the main daughter nuclei. This can result in the formation of small secondary nuclei or micronuclei.

Micronuclei can be formed in a wide variety of cell types, but in this test system bone marrow erythrocytes are observed because micronuclei can easily be detected in this cell type, since the nucleus proper is extruded during maturation.

A few hours after their last division is completed, erythroblasts expel their nuclei and become polychromatic erythrocytes. The term polychromatic is derived from the reaction of the cell with Romanovsky stains; residues of nucleic acids remain for a short time after the expulsion of the nucleus causing the cell to stain a blue-grey colour, whereas the mature erythrocyte appears pink.

Polychromatic erythrocytes are useful for the detection of clastogenic chemicals because they persist for only 24 hours before maturing into normochromatic erythrocytes. Consequently, any micronuclei in these cells will have been produced at the last mitotic division and their formation will be due to the effects of the chemical in the preceding 48 hours.

The clastogenic potential of TDI was assessed in the micronucleus assay, following its administration by the inhalation route for a single 6 hour period. The established clastogen, vinyl chloride, was used as a positive control in order to demonstrate the sensitivity of the test system.

The original data from this study are stored in the ICI Central Toxicology Laboratory (CTL) Archives and the final report in the CTL Report Centre.

The experimental phase of the study was carried out between 25 January 1989 and 7 October 1989. The slides were analysed between 14 April 1989 and 29 April 1991.

2. EXPERIMENTAL PROCEDURES

2.1 Test Sample

The test sample of TDI was submitted for testing by the International Isocyanate Institute as a colourless liquid with a certified purity of 99.9% v/v and a 2,4 isomer content of 80.2% w/w. The sample was re-analysed following completion of the study and a certified purity of 100% v/v and 2,4 isomer content of 79.7% w/w were reported. These values therefore indicate the stability of the test material over a period of greater than 3 years. The sample had Sponsor Reference AT/88/68, was given the CTL reference number Y00140/007 and was stored at 0-4°C in the dark until required.

2.2 Control Chemicals

The positive control, vinyl chloride, was supplied by Cambrian Chemicals, Croydon, UK and was given the CTL reference number Y00204/004.

2.3 Generation of Atmospheres

Atmospheres of TDI and vinyl chloride were generated and analysed by the methods described in Appendices A and B.

2.4 Animals and Husbandry

Male and female C57BL/6JfCD-1/Alpk mice in the age range of 6-12 weeks were used for Phase I, mice in the age range of 8-12 weeks were used for Phase II and mice in the age range of 8-9 weeks were used for Phase III of the study.

The animals were supplied by the Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK.

On arrival the mice were housed by sex with between 5 and 10 per cage on single sided wire mesh mouse cage racks or in the long-term inhalation chambers and given food, Porton Combined Diet [PCD] (supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK; Appendix D) and filtered tap water ad libitum.

The animal rooms were maintained at a temperature range of approximately 21°C and within a relative humidity (RH) range of 45-55%. Excursions outside this RH range were noted throughout the study but this is considered not to affect the integrity of the study. The RH was measured using a Kew Pattern wet and dry bulb hygrometer. Lighting was controlled to provide 12 hours artificial light followed by 12 hours darkness. The animal room was under positive pressure with respect to the access corridor and had 20-30 air changes per hour.

2.5 Test Method

2.5.1 Exposure Chambers: All animals were exposed whole-body in 17.0 litre all-glass dessicators fitted with perforated aluminium bases. One chamber was used per sex per concentration group.

2.5.2 Generation Systems:

a) TDI - Phase I

TDI atmospheres for Phase I of the study were generated by passing clean dry air from the laboratory air supply via a flow controller and a flow meter to a jacketed bubbler containing the test material (Appendix A). The bubbler was heated by a flow of warm air at a temperature controlled by a thermocirculator. The generation air passed through the bubbler picking up vapourised test material and was split into two streams, each stream being passed to one of the 2 exposure chambers being used.

Two streams of dilution air were each passed through flow controllers and flow meters. Each of the dilution streams then joined a generation stream prior to entry to the exposure chamber. The diluted test material stream was then passed through the exposure chamber and was subsequently vented into a fume cupboard. Air flows were monitored continuously using flowmeters (KDG Flowmeters, Burgess Hill, Sussex, UK) and were recorded at approximately 30 minute intervals during the exposure periods.

b) TDI - Phase II and Phase III

The atmospheres for Phases II and III of the study were generated using the system described above except that flow controllers were not fitted into the system and as only single chambers were supplied from each generation system, only one dilution stream was used.

c) Vinyl Chloride

Vinyl chloride was extracted from the cylinder and mixed with compressed air at a flow rate to allow generation of 50000ppm. Both the air and vinyl chloride flow rates were monitored using in-line flow meters with needle valves.

Flow diagrams and more specific details of the generation systems for both TDI and vinyl chloride are detailed in Appendix A.

2.5.3 Atmosphere Analysis: Actual concentrations of TDI and vinyl chloride were measured approximately every 30-40 minutes during the exposure period, using a gas chromatography system. The details of these methods and results are shown in Appendix B.

2.5.4 Study Design: Phase I involved the determination of the median lethal concentration calculated on the deaths over a four-day observation period (MLC) using a single 6 hour inhalation exposure as shown in Appendix E. Prior to, and after exposure, the treated animals were housed in the long-term inhalation chambers.

After acclimatisation, the mice for Phase II were randomly distributed to the groups using a latin square method until each group contained the appropriate number of mice. The animals were uniquely identified by cage cards and by tail numbering according to the study design shown in Appendix F. Prior to exposure all animals were weighed (Appendix I).

In Phase II, male and female animals were given a single 6 hour whole-body inhalation exposure to clean, dry air, vinyl chloride at a target concentration of 50000ppm or TDI at target concentrations of 7.5 and 11.9ppm for females and 11.8 and 18.9ppm for males. Prior to, and after exposure, the treated animals were housed in groups of 5 animals in an animal holding room adjacent to the inhalation laboratory. Clinical observations were recorded at approximately 30 minute intervals where possible during the exposure period. Subsequent observations were made at least once daily following exposure.

The lower concentrations were used to ensure a test result in the event of excessive deaths in the top concentration group, and to allow the observation of a concentration response, should the higher concentration give a positive result. Bone marrow smears were prepared as detailed below 24, 48 and 72 hours after the end of the exposure periods.

Following the results of the cytogenetic analysis of Phase II, Phase III was conducted by giving male and female animals a single 6 hour whole-body inhalation exposure to clean, dry air, vinyl chloride at a target

concentration of 50000ppm or TDI at target concentrations of 3.7, 7.5 and 11.9ppm for females and 5.9, 11.8 and 18.9 for males. Prior to and after exposure, the treated animals were housed in groups of 5 animals in an animal holding room adjacent to the inhalation laboratory. Clinical observations were recorded at approximately 30 minute intervals where possible during the exposure period. Subsequent observations were made at least once daily following exposure. Bone marrow smears were prepared 24 hours after the end of the exposure periods.

An additional 5 males were exposed to TDI at 18.9ppm and an additional 5 females were exposed to TDI at 11.9ppm. These animals were used to replace any animals exposed at that concentration that were found dead or killed in extremis prior to their scheduled termination time. The additional mice not used for this study were killed and discarded without smear preparation after the 24 hour kill.

2.5.5 Summary of Methodology: Bone marrow smears were prepared 24, 48 and 72 hours after the end of the exposure periods in Phase II and 24 hours after the end of the exposure period in Phase III. The preparations were stained with polychrome methylene blue and eosin to visualise the various cell types. One thousand polychromatic erythrocytes per slide were originally evaluated for the presence of micronuclei. An additional 2000 polychromatic erythrocytes were also evaluated for the presence of micronuclei from all slides from male animals exposed to the air control or TDI 24 hours after exposure and female animals exposed to the air control or TDI 24 and 48 hours after exposure in Phase II. In addition 1000 erythrocytes were counted to determine the percentage of polychromatic erythrocytes in the total erythrocyte population. This provides an indication of any cytotoxicity in the bone marrow. Detailed methodology is shown in Appendix C.

2.6 Statistical Analyses

The incidence of micronucleated polychromatic erythrocytes and percentage polychromatic erythrocytes in the erythrocyte sample, were considered by analysis of variance, regarding each combination of sampling time,

concentration and sex as a separate group. The results were examined to determine whether any differences between air control and TDI treated groups were consistent between sexes and across sampling times. The data from the extended counts were similarly analysed as an independent database and also after combination with the original counts. All analyses were carried out after calculating the average number of micronuclei per 1000 polychromatic erythrocytes. The values for micronucleated polychromatic erythrocytes were transformed using a natural logarithmic transformation, to stabilise the variance, before analysis.

All analyses were carried out using the GLM procedure in SAS (1985). Unbiased estimates of the group means were provided by the least square means (LSMEANS option in SAS) but for simplicity standard means are presented. Each treatment group mean was compared with the air control group mean at the corresponding sampling time using a one-sided Student's t-test based on the error mean square in the analysis.

3. RESULTS

3.1 Atmosphere Analysis

The TDI and vinyl chloride group mean atmosphere concentrations for Phases I, II and III are detailed in Tables 13, 14 and 15 respectively, whilst the methods and individual results are detailed in Appendix B. Examination of peak areas of the gas chromatograph confirms that the ratio of 2,4 to 2,6 TDI in the exposure chambers approximated closely to the expected ratio of 80:20. There was no TDI detected in control group atmospheres and no TDI or vinyl chloride were detected in the room air samples.

3.2 Phase I - MLC Determination

Groups of 5 male and 5 female mice were exposed to TDI at target concentrations of 7, 10, 15, 20 and 30ppm. From the resultant mortalities

the MLC over a four day observation period was calculated by logistic regression as 14.1ppm for females and by linear log interpolation as 19.0ppm for males. Atmosphere concentrations of 11.8 and 18.9ppm for males and 7.5 and 11.9ppm for females were used in Phase II of the study. In both cases these concentrations were selected to represent 50 and 80% respectively of the median lethal concentration (MLC). Due to an error in the original calculation of the MLC values the target concentrations used actually represented 62 and 99% of the MLC in males and 53 and 84% of the MLC in females.

3.3 Phase II and Phase III - Micronucleus Test

The data for individual animals are shown in Appendices G and H and the group data are summarised in Tables 1 to 12.

In Phase II of the study clinical signs were recorded for mice exposed to TDI as follows: male mice exposed to TDI at the target concentration of 11.8ppm had a reduced response to stimulus throughout the exposure period, subdued nature, increased breathing depth, reduced breathing rate and piloerection, whereas male mice exposed to TDI at 18.9ppm had no visible response to stimulus, very subdued nature, hunched posture, reduced breathing rate and increased breathing depth. The males exposed to TDI at 18.9ppm were also noted to be subdued the day following exposure. In addition, one male exposed to TDI at 18.9ppm was found dead in its cage approximately 48 hours after exposure.

Female mice exposed to TDI at the target concentration of 7.5 and 11.9ppm exhibited reduced response to stimulus, reduced breathing rate and increased breathing depth during exposure. In addition the females exposed to the target concentration of 11.9ppm exhibited hunched posture and piloerection during exposure. After exposure females exposed to TDI at 7.5ppm exhibited hunched posture, subdued nature and piloerection, whereas those exposed at 11.9ppm exhibited clinical signs including subdued nature, hunched posture, piloerection and reduced temperature. In addition, one female exposed to TDI at 11.9ppm was found dead in its cage 24 hours after the end of the exposure period.

Males exposed to vinyl chloride were noted to have a slightly subdued nature, a reduced response to stimulus and hunched posture during exposure, and one male exhibited a subdued nature the day following exposure. Females exposed to vinyl chloride were noted to be exhibiting a subdued nature, hunched posture, piloerection, reduced response to stimulus, reduced breathing rate and increased breathing depth during the exposure period and hunched posture, subdued nature and piloerection after exposure.

Small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the air control values, were observed in females 24 hours after being exposed at the target concentration of 7.5ppm and 24 and 48 hours after being exposed at the target concentration of 11.9ppm (Table 2). These increases were small and not concentration-related.

Extended analysis of a further 2000 polychromatic erythrocytes from these animals and the female air control animals at the 24 and 48 hour time points was conducted. No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes were observed in these extended counts (Table 5). However, when the original and extended analyses were combined prior to statistical analysis small but statistically significant increases were observed in females 24 hours after being exposed at both target concentrations (Table 6).

Small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the air control values, were observed in males 24 hours after being exposed at the target concentrations of 11.8 and 18.9ppm but there was no clear concentration-response relationship (Table 1). Extended analysis of a further 2000 polychromatic erythrocytes from these animals and the male air control animals at the 24 hour time point was conducted. A small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes was observed only at the lower target concentration (11.8ppm) in these extended counts (Table 3) and when the original and extended analyses were pooled prior to statistical analysis (Table 4).

In order to further investigate the increases observed in both males and females exposed to TDI and the lack of concentration-response relationships observed a second assay was conducted. Groups of 5 male mice were exposed to TDI for a 6 hour period by the inhalation route at target concentrations of 5.9, 11.8 and 18.9ppm and groups of 5 female mice were similarly exposed to TDI at target concentrations of 3.7, 7.5 and 11.9ppm. In both cases these concentrations were selected to represent the concentrations used in the first study with an additional lower concentration to investigate any concentration-response relationships. Due to error in the original calculation of the MLC values, the target concentrations used actually represented approximately 31, 62 and 99% of the MLC in males and 26, 53 and 84% of the MLC in females. Bone marrow samples were taken 24 hours after the end of the exposure period for all concentrations.

Adverse reactions to treatment was recorded for mice exposed to TDI. Clinical signs recorded for male mice exposed to TDI at 5.9, 11.8 and 18.9ppm were reduced response to stimulus, subdued nature and decreased breathing rate during exposure, although due to misting of the inside of the exposure chamber difficulty was experienced in carrying out the clinical observations on the 11.8 and 18.9ppm concentration groups. In addition, 4 males exposed to TDI at 11.8ppm were found dead in their cages and the remaining male was killed in extremis. One male exposed to TDI at 18.9ppm was killed in extremis.

Clinical signs recorded for female mice exposed to TDI at 3.7, 7.5 and 11.9ppm included reduced response to stimulus and reduced breathing rate. In addition, females exposed to TDI at 7.5 and 11.9ppm exhibited hunched posture and little movement although difficulty was experienced in carrying out the clinical observations due to misting of the exposure chambers. In addition, six females exposed to TDI at 11.9ppm were found dead in their cages following exposure to TDI.

Males exposed to vinyl chloride were noted to have a reduced response to stimulus, piloerection and hunched posture whereas females exposed to vinyl chloride exhibited a hunched posture and reduced response to stimulus.

In this second study high levels of lethality were observed at the 11.8ppm (62% MLC) concentration in males and the 11.9ppm (84% MLC) concentration in females and therefore the slides from the males exposed at the 5.9ppm target concentration and the females exposed at the 3.7 and 7.5ppm target concentrations only were analysed. The maximum concentration in each case is considered to represent a maximum tolerated concentration (MTC) in this second study.

No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, compared to the air control values, were observed in the males exposed to TDI (Table 9).

Small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the air control values, were observed in females 24 hours after being exposed at both the 3.7 and 7.5ppm target concentrations (Table 10). These increases were small and the values fell within the range of female air control values reported in this study.

Statistically significant decreases in the percentage of polychromatic erythrocytes were observed in both males (24 and 48 hours; 18.9ppm; Table 7) and females (24 hours; 11.9ppm; Table 8) on the first study and in females exposed at the 7.5ppm target concentration in the second study.

The test system positive control, vinyl chloride, induced statistically and biologically significant increases in the incidence of micronucleated polychromatic erythrocytes in both male and female animals at the 24 hour sampling time on both studies (Tables 1, 2, 9 and 10).

4. DISCUSSION

The criteria for a valid test system as laid down by OECD Guideline 474 (1983) for the conduct of micronucleus studies, are that the positive control substance should induce a significant elevation in micronucleated

polychromatic erythrocytes compared to the vehicle control values, and that the test compound should be tested at a level that causes a decrease in the percentage of polychromatic erythrocytes (indicating a cytotoxic effect on the bone marrow) or at the maximum tolerated dose level.

The study satisfies these criteria in that TDI was tested in excess of 80% of a median lethal concentration (MLC), a concentration which also induced adverse reactions to treatment. Consideration of the percentage of polychromatic erythrocytes showed statistically significant decreases, compared to the air control values, in both males and females in the first study and in females in the second study. These decreases may indicate that TDI or a metabolite has induced a cytotoxic response in the bone marrow resulting in a depression of cell proliferation. The positive control substance, vinyl chloride, gave a statistically significant and biologically meaningful increase in micronucleated polychromatic erythrocytes, compared to air control values, in both male and female mice in both studies.

Small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the air control values, were observed in both males and females exposed to TDI in the first study. The increases at the 24 hour time point were confirmed by extended analysis of the slides and a second assay was conducted to clarify the increases observed.

No statistically or biologically significant increases were observed in the males in the second study, and although small but statistically significant increases were observed in the females the values fell within the range of female air control values reported in this study. It is therefore considered that the increases observed in the second study are due to a low control value rather than to any effect of TDI. The increases are therefore considered not to be biologically significant.

In summary, although increases in the incidence of micronucleated polychromatic erythrocytes were observed in both males and females exposed to TDI these increases were small, not concentration-related and were not

reproducible at concentrations limited by lethality in a repeat study. It is therefore considered that the increases observed are of no biological significance and do not indicate any clastogenic activity of TDI in the mouse bone marrow micronucleus assay.

5. CONCLUSION

TDI, under the conditions of test, is not clastogenic in the mouse micronucleus test.

6. REFERENCES

OECD Guidelines for Testing of Chemicals (1983). Genetic Toxicology: Micronucleus Test - No. 474.

SAS Institute Inc, SAS Users Guide (1985). Statistics, Version 5 Edition, Cary, NC: SAS Institute Inc.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 1

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT THREE SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

ORIGINAL COUNTS

Group	Compound	Conc (ppm)	Mean Incidence of MPE/1000 PE ± SD		
			24 hours	48 hours	72 hours
11	Air Control	-	2.0 ± 1.2	1.4 ± 0.9	2.0 ± 1.6
12	Vinyl Chloride	50000	15.8 ± 7.3**		
13	Toluene di-isocyanate	11.8	7.4 ± 4.5**		
15	Toluene di-isocyanate	18.9	4.4 ± 2.0*	1.8 ± 2.9 (4)	1.8 ± 0.8

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.
Conc = concentration

* Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided) on transformed data.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

All means based on 5 observations, except where indicated in parentheses.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 2

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT THREE SAMPLING TIMES

GROUP MEAN ANIMAL DATA - FEMALES

ORIGINAL COUNTS

Group	Compound	Conc (ppm)	Mean Incidence of MPE/1000 PE ± SD		
			24 hours	48 hours	72 hours
17	Air Control	-	0.4 ± 0.9	0.6 ± 0.6	1.4 ± 0.6
18	Vinyl Chloride	50000	8.6 ± 1.5**		
19	Toluene di-isocyanate	7.5	4.0 ± 1.4**		
20	Toluene di-isocyanate	11.9	1.8 ± 1.5*	2.0 ± 1.4*	0.5 ± 0.6 (4)

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.
Conc = concentration

* Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided) on transformed data.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

All means based on 5 observations, except where indicated in parentheses.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 3

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT ONE SAMPLING TIME

GROUP MEAN ANIMAL DATA - MALES

EXTENDED COUNTS+

Group	Compound	Conc (ppm)	Mean Incidence of MPE/1000 PE ± SD
			24 hours
11	Air Control	-	2.8 ± 1.1
13	Toluene di-isocyanate	11.8	5.9 ± 2.1**
15	Toluene di-isocyanate	18.9	1.9 ± 1.1

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.
Conc = concentration

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

All means based on 10 observations (2 observations from each of 5 animals per group).

+ Means based on the assessment of an additional 2000 polychromatic erythrocytes per animal.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 4

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT ONE SAMPLING TIME

GROUP MEAN ANIMAL DATA - MALES

COMBINED ORIGINAL AND EXTENDED COUNTS+

Group	Compound	Conc (ppm)	Mean Incidence of MPE/1000 PE ± SD
			24 hours
11	Air Control	-	2.5 ± 1.2
13	Toluene di-isocyanate	11.8	6.4 ± 3.0**
15	Toluene di-isocyanate	18.9	2.7 ± 1.8

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.
Conc = concentration

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

All means based on 15 observations (3 observations from each of 5 animals per group).

+ Means based on the assessment of 3000 polychromatic erythrocytes per animal.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 5

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - FEMALES

EXTENDED COUNTS+

Group	Compound	Conc (ppm)	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
17	Air Control	-	1.4 ± 1.6	1.3 ± 1.1
19	Toluene di-isocyanate	7.5	2.3 ± 2.1	
20	Toluene di-isocyanate	11.9	2.0 ± 1.4	1.5 ± 1.3

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.
Conc = concentration

All means based on 10 observations (2 observations from each of 5 animals per group).

+ Means based on the assessment of an additional 2000 polychromatic erythrocytes per animal.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 6

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - FEMALES

COMBINED ORIGINAL AND EXTENDED COUNTS+

Group	Compound	Conc (ppm)	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
17	Air Control	-	1.1 ± 1.4	1.1 ± 1.0
19	Toluene di-isocyanate	7.5	2.9 ± 2.0**	
20	Toluene di-isocyanate	11.9	1.9 ± 1.4*	1.7 ± 1.3

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.
Conc = concentration

* Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided) on transformed data.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

All means based on 15 observations (3 observations from each of 5 animals per group).

+ Means based on the assessment of 3000 polychromatic erythrocytes per animal.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 7

MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES
± STANDARD DEVIATION (SD) AT THREE SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Group	Compound	Conc (ppm)	Mean % Polychromatic Erythrocytes ± SD		
			24 hours	48 hours	72 hours
11	Air Control	-	38.3 ± 3.9	40.7 ± 2.1	41.1 ± 4.4
12	Vinyl Chloride	50000	36.6 ± 7.7		
13	Toluene di-isocyanate	11.8	38.8 ± 7.9		
15	Toluene di-isocyanate	18.9	27.1 ± 7.4**	29.8 ± 13.5** (4)	35.3 ± 6.0

SD = standard deviation.
Conc = concentration

** Statistically significant decrease in the percentage of polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided).

All means based on 5 observations, except where indicated in parentheses.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 8

MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES
± STANDARD DEVIATION (SD) AT THREE SAMPLING TIMES

GROUP MEAN ANIMAL DATA - FEMALES

Group	Compound	Conc (ppm)	Mean % Polychromatic Erythrocytes ± SD		
			24 hours	48 hours	72 hours
17	Air Control	-	41.7 ± 5.8	34.7 ± 7.1	37.1 ± 7.3
18	Vinyl Chloride	50000	34.5 ± 7.0*		
19	Toluene di-isocyanate	7.5	39.1 ± 5.0		
20	Toluene di-isocyanate	11.9	34.0 ± 3.1*	29.5 ± 4.3	33.2 ± 7.1 (4)

SD = standard deviation.
Conc = concentration

* Statistically significant decrease in the percentage of polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided).

All means based on 5 observations, except where indicated in parentheses.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 9

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT ONE SAMPLING TIME

GROUP MEAN ANIMAL DATA - MALES

REPEAT STUDY

Group	Compound	Conc (ppm)	Mean Incidence of MPE/1000 PE ± SD
			24 hours
21	Air Control	-	1.2 ± 0.5
22	Vinyl Chloride	50000	8.8 ± 3.1**
23	Toluene di-isocyanate	5.9	2.0 ± 1.9

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.
Conc = concentration

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

All means based on 5 observations.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 10

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT ONE SAMPLING TIME

GROUP MEAN ANIMAL DATA - FEMALES

REPEAT STUDY

Group	Compound	Conc (ppm)	Mean Incidence of MPE/1000 PE ± SD
			24 hours
21	Air Control	-	0.2 ± 0.5
22	Vinyl Chloride	50000	8.4 ± 6.8**
24	Toluene di-isocyanate	3.7	1.2 ± 0.8*
26	Toluene di-isocyanate	7.5	1.4 ± 0.9*

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.
Conc = concentration

* Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided) on transformed data.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

All means based on 5 observations.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 11

MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES
± STANDARD DEVIATION (SD) AT ONE SAMPLING TIME

GROUP MEAN ANIMAL DATA - MALES

REPEAT STUDY

Group	Compound	Conc (ppm)	Mean % Polychromatic Erythrocytes ± SD
			24 hours
21	Air Control	-	48.4 ± 3.3
22	Vinyl Chloride	50000	45.1 ± 6.6
23	Toluene di-isocyanate	5.9	46.1 ± 4.1

SD = standard deviation.
Conc = concentration

All means based on 5 observations.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 12

MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES
± STANDARD DEVIATION (SD) AT ONE SAMPLING TIME

GROUP MEAN ANIMAL DATA - FEMALES

REPEAT STUDY

Group	Compound	Conc (ppm)	Mean % Polychromatic Erythrocytes ± SD
			24 hours
21	Air Control	-	47.3 ± 2.8
22	Vinyl Chloride	50000	44.6 ± 5.5
24	Toluene di-isocyanate	3.7	43.9 ± 1.0
26	Toluene di-isocyanate	7.5	40.1 ± 8.0*

SD = standard deviation.
Conc = concentration

* Statistically significant decrease in the percentage of polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided).

All means based on 5 observations.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 13

GROUP MEAN ATMOSPHERE CONCENTRATIONS FOR TOLUENE DI-ISOCYANATE ANALYSIS OVER A SIX-HOUR EXPOSURE PERIOD

PHASE I

Group	Target Concentration (ppm)	Actual Concentration ppm ± SD
1 ♂	7	7.10 ± 0.51
1 ♀	7	7.43 ± 1.43
2 ♂	10	10.02 ± 2.25
2 ♀	10	10.86 ± 1.81
3 ♂	20	a
3 ♀	20	a
4 ♂	30	28.96 ± 4.15
4 ♀	30	29.97 ± 3.04
5 ♂	20	21.45 ± 3.13
5 ♀	20	20.15 ± 2.66
6 ♂	15	16.78 ± 1.86
6 ♀	15	16.17 ± 0.68

SD - standard deviation

a - generation problems, not used in this study.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 14

GROUP MEAN ATMOSPHERE CONCENTRATIONS FOR TOLUENE DI-ISOCYANATE AND VINYL CHLORIDE ANALYSIS OVER A SIX HOUR EXPOSURE PERIOD

PHASE II

Group	Target Concentration (ppm)	Actual Concentration ppm ± SD
11 ♂	0	0
12 ♂	50000	49529 ± 1778
13 ♂	11.8	11.22 ± 1.57
15 ♂	18.9	17.90 ± 2.82
17 ♀	0	0
18 ♀	50000	50286 ± 2474
19 ♀	7.5	7.9 ± 1.2
20 ♀	11.9	11.4 ± 0.9

SD - standard deviation

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

TABLE 15

GROUP MEAN ATMOSPHERE CONCENTRATIONS FOR TOLUENE DI-ISOCYANATE AND VINYL CHLORIDE ANALYSIS OVER A SIX HOUR EXPOSURE PERIOD

PHASE III

Group	Target Concentration (ppm)	Actual Concentration ppm ± SD
21 ♂	0	0
21 ♀	0	0
22 ♂	50000	47287 ± 2813
22 ♀	50000	48570 ± 2255
23 ♂	5.9	5.97 ± 0.88
24 ♀	3.7	3.59 ± 0.64
25 ♂	11.8	11.71 ± 0.63
26 ♀	7.5	8.74 ± 1.18
27 ♂	18.9	19.89 ± 3.00
28 ♀	11.9	12.82 ± 0.99

SD - standard deviation

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX A

TOLUENE DI-ISOCYANATE GENERATION SYSTEM AND
VINYL CHLORIDE GENERATION SYSTEM

PHASE I

Clean dry air from the laboratory air supply was passed via a flow controller and a flow meter to a-jacketed bubbler containing the test material. The bubbler was heated by a flow of warm water at a temperature controlled by a thermocirculator. The generation air passed through the bubbler picking up vapourised test material and was split into two streams, each stream going to one of the 2 exposure chambers.

Two streams of dilution air were each passed through flow controllers and flow meters. Each of the dilution streams then joined a generation stream prior to the exposure chamber (Figure 1). The diluted test material stream then passed through the exposure chamber and was vented to the fume cupboard.

PHASE II AND PHASE III

The generations for Phase II and Phase III were as for Phase I except that flow controllers were not fitted into the system and as two chambers were not supplied from one generation system, there was only one dilution stream. The system is shown diagramatically in Figure 2.

APPENDIX A - continued

TOLUENE DI-ISOCYANATE GENERATION SYSTEM AND
VINYL CHLORIDE GENERATION SYSTEM

CONTROL AIR (PHASE II AND III)

Clean air from a compressed air line was passed through a flow meter to the exposure chamber which was located on a fume cupboard in a room separate from the inhalation bays. The exhaust was vented to the fume cupboard (Figure 3).

VINYL CHLORIDE (PHASE II AND III)

Vinyl chloride was removed from the cylinder through the cylinder head regulator to a flow meter. Dilution air from a compressed air line was passed through a flow meter to join the vinyl chloride system. The diluted vinyl chloride stream was then passed to the exposure chamber and the chamber exhaust was vented to the fume cupboard (Figure 4).

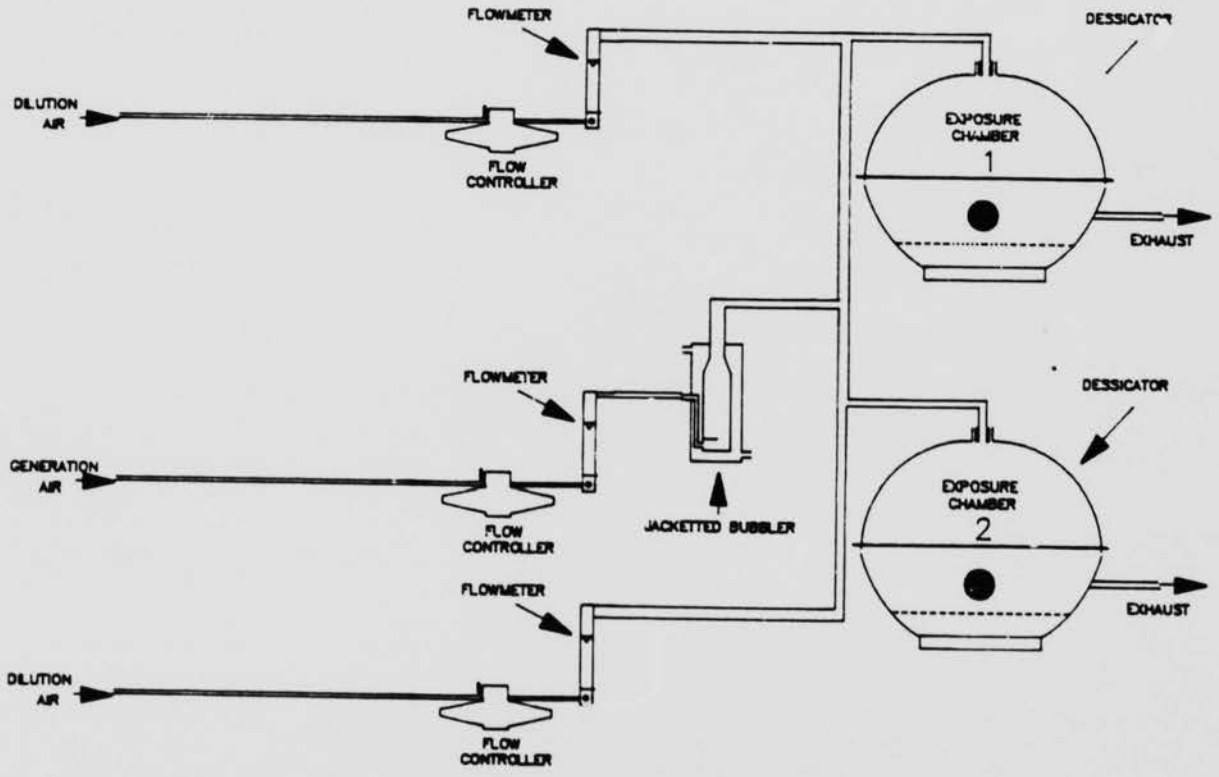
TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX A - continued

TOLUENE DI-ISOCYANATE GENERATION SYSTEM AND
VINYL CHLORIDE GENERATION SYSTEM

FIGURE 1

PHASE 1 TDI EXPOSURE SYSTEM



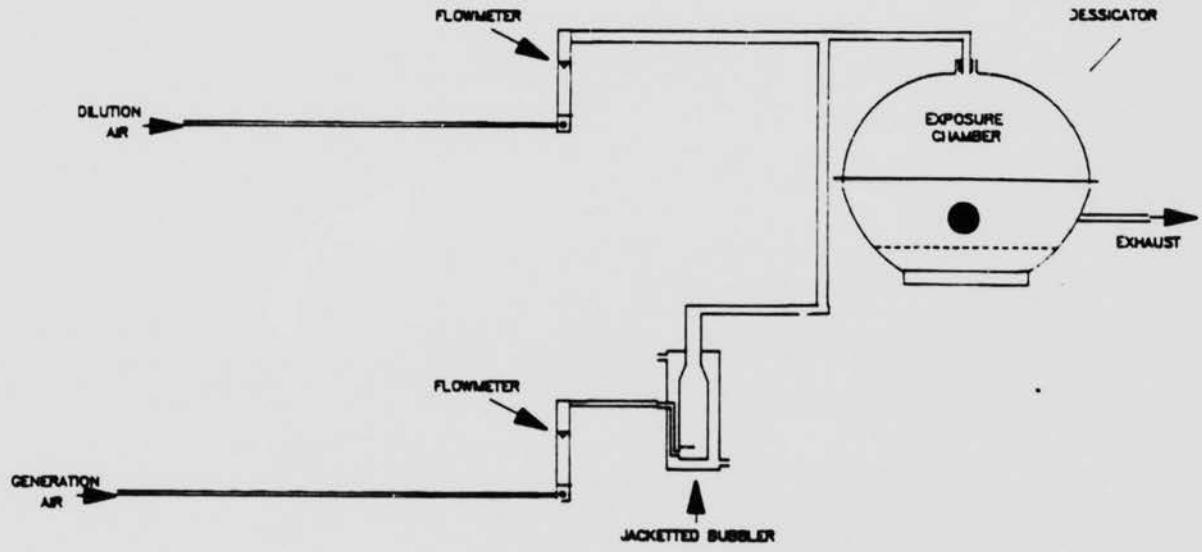
TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX A - continued

TOLUENE DI-ISOCYANATE GENERATION SYSTEM AND
VINYL CHLORIDE GENERATION SYSTEM

FIGURE 2

PHASE II AND III TDI EXPOSURE SYSTEM



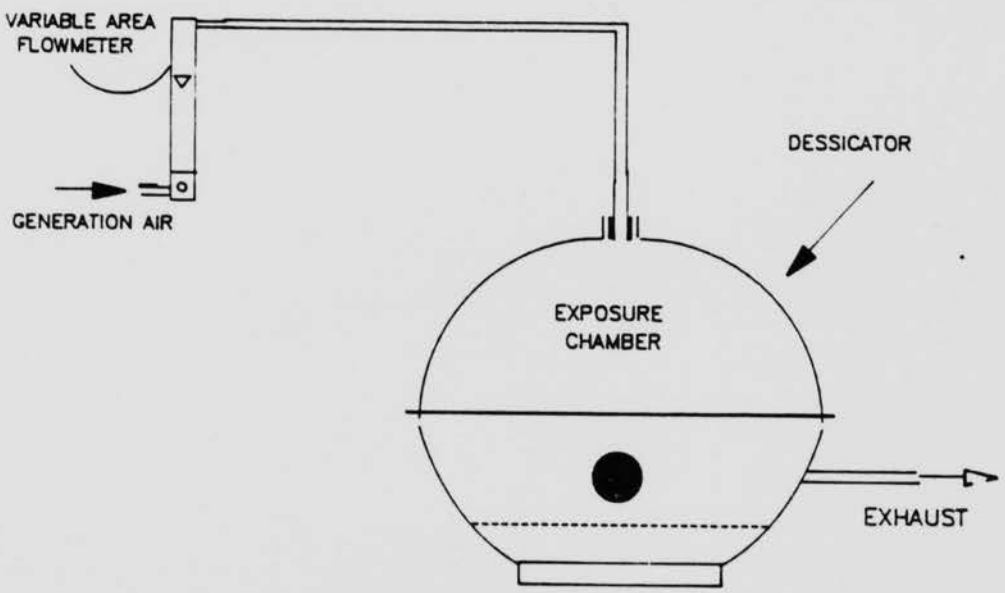
TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX A - continued

TOLUENE DI-ISOCYANATE GENERATION SYSTEM AND
VINYL CHLORIDE GENERATION SYSTEM

FIGURE 3

AIR CONTROL EXPOSURE SYSTEM



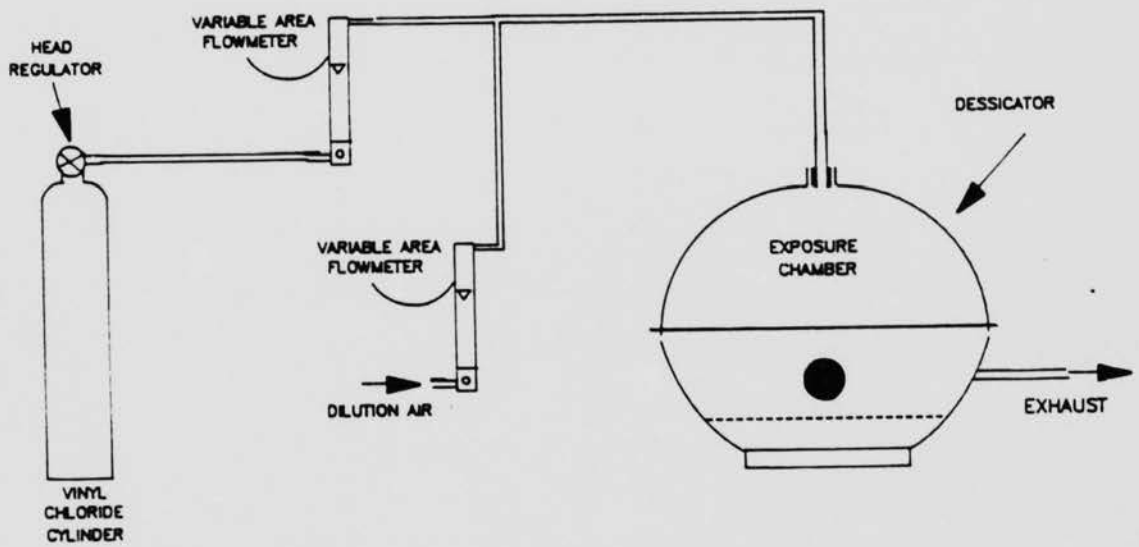
TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX A - continued

TOLUENE DI-ISOCYANATE GENERATION SYSTEM AND
VINYL CHLORIDE GENERATION SYSTEM

FIGURE 4

VINYL CHLORIDE EXPOSURE SYSTEM



TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

1. METHOD FOR THE DETERMINATION OF TOLUENE DI-ISOCYANATE IN SAMPLE ATMOSPHERES

METHOD SUMMARY

Samples of the atmospheres were collected by passing through a sintered impinger with a trapping reagent. The sample was rotary evaporated to dryness, re-dissolved in dichloromethane and analysed by high performance liquid chromatography.

CHEMICALS

Hexane, (HPLC grade)
Dichloromethane, (HPLC grade)
Methanol, (Analar grade)
N-(4-nitrobenzyl)-N-propylamine hydrochloride - Aldrich Chemical Co Inc
Distilled water
Sodium hydroxide, (Analar grade)

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

Preparation of Nitro-Reagent (Standard)

N-(4-nitrobenzyl)-N-propylamine hydrochloride (592mg) was dissolved in 20ml of distilled water in a 100ml beaker. The solution was transferred to a suitably sized separating funnel and 26ml 1N sodium hydroxide added to precipitate the free amine. The free nitro-reagent was extracted with 5 portions of 50ml dichloromethane and made to a final volume of 500ml (5.15×10^{-3}M).

Preparation of Nitro-Reagent (Sample absorber solution)

As above but taking 300mg of N-(4-nitrobenzyl)-N-propylamine hydrochloride and using toluene in place of dichloromethane. The prepared solution (2.6×10^{-3}M) was diluted 10 times to prepare the nitro-absorber solution (2.6×10^{-4}M).

CALIBRATION STANDARDS

Approximately 0.04g (Phase II) or 0.06g (Phase III) toluene di-isocyanate (TDI), (CTL Reference Y00140/007) was accurately weighed into a tared and stoppered 100ml volumetric flask and nitro-reagent (standard) added to give a nominal concentration of 0.4mg/ml (Phase II) or 0.6mg/ml (Phase III) (stock solution).

Portions of the stock solution were diluted to give standards to cover the range 0-42µg/ml (Phase II) or 6-26µg/ml (Phase III).

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

PROCEDURE

(1) Sample Preparation

Samples were taken by drawing the atmosphere through a sample impinger tube containing 20ml nitro-reagent (sample absorber solution) at 1 litre/minute for 1 minute. The samples were taken to dryness by rotary evaporation at <40°C and 5ml of dichloromethane added.

(2) Room Air

As above but diluted with 1ml of dichloromethane (five samples per 6 hour exposure period were taken).

(3) Liquid Chromatographic Analysis

Pump	: SA6410B (Severn Analytical Ltd)
Detector	: SA6500 (Severn Analytical Ltd)
Wavelength	: 255nm
Column	: Spherisorb silica S3W, 3µm particle size (Hichrom Ltd)
Column Dimensions	: 50 x 4.9mm id
Mobile Phase	: Hexane:dichloromethane:methanol 140:100:5 v/v
Flow Rate	: 2.0ml/min
Injector	: Rheodyne valve
Injection Volume	: 20µl
Data Handling	: Chromatopac C-R3A (Shimadzu Corp)

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

The analysis system was calibrated and the linearity checked with the working standards. During the study analysis the calibration of the analysis system was checked with a 7.26µg/ml or 21.1µg/ml standard for the low or high dose groups respectively (Phase II) and a 13.06µg/ml (9.01ppm) standard interspersed between samples at regular intervals (Phase III). A response factor was calculated and input into the integrator to enable direct calculation of results in ppm. The samples were manually injected using a 100µl syringe and a Rheodyne valve equipped with a 25µl sample loop.

$$\text{Response factor} = \frac{\text{amount}}{\text{area}} = \frac{\text{Cstd}}{\text{Astd}}$$

Where:

Cstd = Concentration of standard (7.26 or 21.1µg/ml [Phase II] or 13.06µg/ml [Phase III]).

Astd = Area of peak given by the standard

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

(4) Calculation of Results

Analysed atmosphere concentration.

$$Ac = \frac{Cs \times Mv \times V_1}{Va \times Mwt \times 1000}$$

Where:

Ac = Analysed atmosphere concentration (ppm)
Cs = Solution concentration (µg/ml)
V_1 = Final sample volume (ml)
Va = Sample volume (1)
Mv = 24000
Mwt = 174

TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

Group	13		15		19		20	
Target Concn. (ppm)	11.8		18.88		7.50		11.92	
Sex	Male		Male		Female		Female	
Sample Number	Time	Concn. (ppm)	Time	Concn. (ppm)	Time	Concn. (ppm)	Time	Concn. (ppm)
1	13.30	12.80	11.14	18.20				
2	13.55	12.53	11.47	13.66				
3	14.50	12.02	12.24	14.87				
4	15.33	11.80	13.01	15.78	11.34	6.7	11.16	a
5	16.27	10.93	13.40	21.59	12.00	6.8	11.28	9.3
6	16.55	10.90	14.37	16.77	12.32	6.8	11.58	9.8
7	17.26	13.47	15.20	21.20	13.05	8.3	12.26	12.6
8	17.56	9.62	16.03	19.93	13.29	7.3	12.59	11.5
9	18.20	8.91	16.33	19.06	13.56	7.1	13.26	12.2
10	18.41	9.26			14.30	6.9	13.50	11.7
11					14.56	10.9	14.28	11.4
12					15.32	8.7	14.55	11.3
13					15.58	8.1	15.27	11.7
14					16.16	7.9	15.51	11.3
15					16.39	8.9	16.12	11.5
16							16.38	12.0
17								
Mean		11.22		17.90		7.9		11.4
SD		1.57		2.82		1.2		0.9

a - sample lost

TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

Group	23		24		25		26		27		28	
Target Concn. (ppm)	5.9		3.7		11.8		7.5		18.9		11.9	
Sex	Male		Female		Male		Female		Male		Female	
Sample Number	Time	Concn. (ppm)	Time	Concn. (ppm)	Time	Concn. (ppm)	Time	Concn. (ppm)	Time	Concn. (ppm)	Time	Concn. (ppm)
1												
2	10.58	5.40	12.20	3.92	11.02	11.71	12.22	10.18	11.05	20.89	12.24	14.08
3	11.25	4.43	12.50	3.65	11.28	11.15	12.52	8.98	11.30	22.02	12.54	12.61
4	12.05	6.53	13.26	3.13	12.07	12.45	13.28	6.85	12.09	23.86	13.30	12.49
5	12.33	6.48	14.02	3.22	12.35	12.51	14.04	10.12	12.37	24.36	14.06	11.10
6	13.05	7.32	a	-	13.07	12.51	a	-	13.09	21.48	a	-
7	13.41	5.92	15.01	2.77	13.43	10.70	15.03	7.41	13.45	15.85	15.05	12.05
8	14.17	4.74	15.32	3.34	14.19	11.14	15.36	8.69	14.21	17.24	15.38	13.04
9	14.49	6.57	16.00	3.24	14.51	11.71	16.02	9.61	14.53	17.71	16.04	13.47
10	15.22	6.24	16.35	4.85	15.24	11.69	16.37	7.75	15.26	17.63	16.39	12.36
11	15.56	6.04	17.15	4.22	15.58	11.51	17.17	9.06	16.00	17.83	17.19	14.18
12												
13												
14												
15												
16												
17												
Mean		5.97		3.59		11.71		8.74		19.89		12.82
SD		0.88		0.64		0.63		1.18		3.00		0.99

a - sample lost

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

2. THE DETERMINATION OF VINYL CHLORIDE IN SAMPLE ATMOSPHERES

METHOD SUMMARY

The test atmospheres were sampled using a gas tight syringe and the samples injected manually via a gas sampling valve into a gas chromatograph equipped with a flame-ionisation detector. The area of the peak due to vinyl chloride was used to calculate the atmosphere concentration in parts per million (ppm), after suitable calibration.

CALIBRATION STANDARDS

Calibration standards were prepared by adding 50ml aliquots of VC, CTL Reference Y00204/004, to 1 litre with air in a gas sampling bag to give a standard of 47620ppm. For the room air analysis 0.15ml of the 47620ppm VC standard was further diluted to 1000ml ie 7.1ppm.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

PROCEDURE

a) Sampling

The test atmospheres were sampled using a gas tight syringe. The samples were injected manually via a gas sampling valve into a gas chromatograph equipped with a flame-ionisation detector. Samples of room air were taken at intervals in a similar way to test atmosphere samples.

b) Typical Gas Chromatographic Conditions
(room air analysis conditions in brackets where different)

Gas Chromatograph	:	Pye Unicam 204 (GCD)
Detector	:	Flame-ionisation
Column Packing	:	0.1% SP1000 on Carbopack C, 80-100 mesh
Column Dimensions	:	1.5m x 4mm ID glass (1.5m x 2mm ID)
Temperatures	:	Column oven - 70°C (65°C)
		Injector - 100°C
		Detector - 150°C
Gases	:	Carrier - Nitrogen, 50ml/min
		Fuel gases - Hydrogen, 30ml/min
		- Air, 300ml/min
Injection Volume	:	0.5ml (1ml)
Data Handling	:	Chromatopack C-R3A, Shimadzu Corp

The chromatographic system was calibrated prior to the study analysis using gaseous standards equivalent to 50000ppm (7ppm room air). The response factor was calculated from the area obtained by injection of a standard.

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

$$\text{Response factor} = \frac{\text{amount}}{\text{area}} = \frac{\text{Cstd}}{\text{Astd}}$$

Where: Astd = Area standard
Cstd = Concentration of gaseous standard (ppm)

$$\text{Concentration of gaseous std (Cstd) (ppm v/v)} = \frac{V_1}{V_2} \times 1000$$

Where: V_1 = Aliquot volume (ml)
V_2 = Dilution volume (litres)

c) Calculation of Results

The results were calculated automatically by the data handling system using the following equation:

Atmosphere concentration (ppm) = Peak area x Response factor

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

Group	12		18	
Target Concn. (ppm)	50000		50000	
Sex	Male		Female	
Sample Number	Time	Concn. (ppm)	Time	Concn. (ppm)
1				
2	10.57	46086	11.00	53998
3	11.30	47174	11.25	53057
4	12.13	46929	11.48	44720
5	12.26	50480	12.10	48714
6	13.04	50445	12.42	49220
7	13.36	50522	13.14	49700
8	13.56	50765	13.40	49201
9	14.19	48991	13.59	49119
10	15.07	50324	14.21	48700
11	15.36	50858	14.46	48628
12	16.15	50815	15.13	52514
13	16.38	50953	15.45	52169
14			16.08	52253
15			16.28	52009
Mean		49529		50286
SD		1778		2474

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX B - continued

ANALYSIS OF LEVELS OF TOLUENE DI-ISOCYANATE
AND VINYL CHLORIDE IN SAMPLE ATMOSPHERES

Group	22		22	
Target Concn. (ppm)	50000		50000	
Sex	Male		Female	
Sample Number	Time	Concn. (ppm)	Time	Concn. (ppm)
1				
2	10.49	48352		
3	11.33	46322	12.13	47253
4	12.15	50267	12.45	47853
5	12.45	51084	13.18	47554
6	13.15	50257	13.56	50632
7	13.46	41635	14.25	47514
8	14.12	48049	14.52	47831
9	14.38	45683	15.25	46860
10	15.05	47077	15.54	52914
11	15.31	47275	16.29	50511
12	15.58	44157	17.05	49894
13			17.33	48358
14				
15				
Mean		47287		48570
SD		2813		2255

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX C

PROCESSING OF BONE MARROW AND CRITERIA FOR IDENTIFICATION OF MICRONUCLEI

The animals were killed by asphyxiation in a rising concentration of carbon dioxide followed by cervical dislocation 24, 48 or 72 hours after receiving a single six hour inhalation exposure to the test material.

a) Femurs were removed and stripped clean of muscle.

b) The iliac end of the femur was removed and a fine paint brush was rinsed in physiological saline, wiped to remove the excess and wetted with a solution of albumin (6% w/v in physiological saline). This was then dipped into the marrow canal and two smears were painted on an appropriately labelled clean, dry microscope slide. This procedure was repeated to give four smears of marrow per slide. The brush was rinsed in physiological saline between animals of the same group, and a separate brush and pot of physiological saline were used between groups to avoid cross contamination.

c) The slides were allowed to dry.

d) The slides were then stained with polychrome methylene blue and eosin using an Ames Hema-Tek staining machine (Hema-Tek, Miles Laboratory Limited, Stoke Court, Stoke Poges, Slough, Berkshire, UK).

e) Slides were coded and scored blind, in numerical slide order.

APPENDIX C - continued

PROCESSING OF BONE MARROW AND CRITERIA FOR IDENTIFICATION OF MICRONUCLEI

f) Initially one thousand polychromatic erythrocytes were examined using x10 or x12.5 eye pieces and a x100 oil immersion objective lens for each animal [slides were assessed starting at the beginning of the smear (label end) and proceeding to the leading edge (Ashby and Mohammed, 1986)] and the number containing micronuclei recorded. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of polychromatic to normochromatic erythrocytes (1000 cells counted). Further analyses of an additional 2000 polychromatic erythrocytes for the presence of micronuclei were carried out as detailed.

Criteria for identification of micronuclei are as described by Schmid (1976):

(i) Spherical (or rounded) with well-defined edges.
(ii) Diameters of not less than approximately 1/20 of a polychromatic erythrocyte diameter.
(iii) Dark purple/dark blue staining.
(iv) Lie in the same plane as the polychromatic erythrocyte in which it is contained (determined by focussing).

References

Ashby J and Mohammed R (1986). Slide preparation and sampling as a major source of variability in the mouse micronucleus assay. Mutation Res 164, 217-235.

Schmid W (1976). The Micronucleus Test for Cytogenetic Analysis. In: A Hollaender (Ed). Chemical Mutagens : Principles and Methods For Their Detection. Vol 4, Plenum, New York 31-43.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX D

COMPOSITION OF PORTON COMBINED DIET (PCD)

Manufacturer - Special Diets Services Ltd, Stepfield, Witham, Essex, UK.

Dietary constituents and a proximate analysis are given below. The diet is prepared to a constant formula, details of which are available on request.

Dietary Constituents	Proximate Analysis (all values calculated to nominal 10% moisture content)	%
Wheat	Crude protein	20.0
Wheat feed	Crude oil	3.0
Oats	Crude fibre	5.0
Maize	Ash	6.9
Barley	Calcium	0.94
Soya bean meal extract	Phosphorus	0.80
British white fish meal		
Skim milk powder (spray dried)		
Yeast (unextracted)		
PCD vitamin and mineral premix		

All batches of PCD diet complied with the following contaminants specification:

Chemical Contaminant	Maximum Permitted Concentration (ppm)	Microbiological Contaminant	Maximum Permitted
Arsenic	1.0	Total viable organisms	2×10^4/ g
Cadmium	0.5		
Lead	3.0		
Mercury	0.1	Mesophilic spores	2×10^4/ g
Selenium	0.5		
DDT (total)	0.1	Salmonella sp	None / g
Dieldrin	0.02		
Heptachlor	0.01	Faecal E coli (Type 1)	None / g
Lindane	0.1		
PCB's (total)	0.05		
		Coliforms	None / g
Fluorine	40		
Nitrite	5.0	Fungal units	200 / g
Nitrate	100		
		Antibiotic activity	None / g
Aflatoxins (total)	0.001		
Malathion	0.5		

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX E

COMPOUND ADMINISTRATION : MLC DETERMINATION

Groups of 5 male and 5 female mice were then given a single 6 hour whole body exposure of TDI at target concentrations of 7, 10, 15, 20 and 30ppm. The results are shown below:-

Group	Treatment	Target Concn. (ppm)	Analysed Concn. (ppm)	Sex	Animal No.	No. of Deaths/ No. of Animals Dosed
1	TDI	7	7.1 7.4	♂ ♀	1-5 6-10	0/5 0/5
2	TDI	10	10.0 10.9	♂ ♀	11-15 16-20	0/5 1/5
3	TDI	a	a	♂ ♀	21-25 26-30	-
4	TDI	30	29.0 30.0	♂ ♀	31-35 36-40	5/5 5/5
5	TDI	20	21.5 20.1	♂ ♀	41-45 46-50	5/5 5/5
6	TDI	15	16.8 16.2	♂ ♀	51-55 56-60	0/5 3/5

a - Generation and analysis problems. Data not included in MLC calculation.

From the resultant mortalities the MLC was calculated by logistic regression as 14.1ppm for females and 19.0ppm for males.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX F

ANIMAL ALLOCATION TO DOSING GROUPS - PHASE II

MALES

Group	Compound	Conc (ppm)	Sex	Animal Numbers/Time of Kill		
				24 hours	48 hours	72 hours
11	Air Control	-	M	101-105	141-145	161-165
12	Vinyl Chloride	50000	M	111-115		
13	TDI	11.8	M	121-125		
15	TDI	18.9	M	131-135	151-155	171-175

M = male

Conc = concentration

NOTE: Animal numbers 106-110, 116-120, 126-130, 136-140, 146-150, 156-160, 166-170 and 176-180 were allocated to female mice which were exposed to TDI as protocolled. Due to a failure to reach the target concentrations during the exposure period this portion of the study was repeated.

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX F - continued

ANIMAL ALLOCATION TO DOSING GROUPS - PHASE II

FEMALES

Group	Compound	Conc (ppm)	Sex	Animal Numbers/Time of Kill		
				24 hours	48 hours	72 hours
17	Air Control	-	F	181-185	186-190	191-195
18	Vinyl Chloride	50000	F	196-200		
19	TDI	7.5	F	201-205		
20	TDI	11.9	F	206-210	211-215	216-220

F = female
Conc = concentration

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX F - continued

ANIMAL ALLOCATION TO DOSING GROUPS - PHASE III

Group	Compound	Conc (ppm)	Sex	Animal Numbers/Time of Kill
				24 hours
21	Air Control	-	M	221-225
			F	226-230
22	Vinyl Chloride	50000	M	231-235
			F	236-240
23	TDI	5.9	M	241-245
24	TDI	3.7	F	246-250
25	TDI	11.8	M	251-255
26	TDI	7.5	F	256-260
27	TDI	18.9	M	261-265
28	TDI	11.9	F	266-270

M = male
F = female
Conc = concentration

TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX G

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

Group	Compound	Dose	Sex	24 HRS					48 HRS					72 HRS					
11	AIR	0 PPM	M	0	2	3	2	3	1	2	2	2	0	4	1	2	0	3
12	VINYL CHLORIDE	50000 PPM	M	18	22	21	14	4										
13	TOLUENE DI-ISOCYANATE	11.8 PPM	M	12	5	12	2	6										
15	TOLUENE DI-ISOCYANATE	18.9 PPM	M	2	3	7	5	5	1	0	0	DEAD	6	2	3	1	1	2
17	AIR	0 PPM	F	0	0	0	2	0	0	1	1	0	1	1	1	1	2	2
18	VINYL CHLORIDE	50000 PPM	F	7	8	8	9	11										
19	TOLUENE DI-ISOCYANATE	7.5 PPM	F	2	6	4	4	4										
20	TOLUENE DI-ISOCYANATE	11.9 PPM	F	0	2	1	2	4	1	1	3	4	1	0	FDED	1	1	0

M - male
F - female

TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX G - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

Group	Compound	Extended Count No. 1 Dose	Sex	24 HRS					48 HRS					72 HRS				
11	AIR	0 PPM	M	1	3	3	4	4										
13	TOLUENE DI-ISOCYANATE	11.8 PPM	M	7	5	6	4	4										
15	TOLUENE DI-ISOCYANATE	18.9 PPM	M	1	1	2	1	1 DEAD										
17	AIR	0 PPM	F	0	0	3	1	0	3	1	2	1	0					
19	TOLUENE DI-ISOCYANATE	7.5 PPM	F	3	2	1	0	3										
20	TOLUENE DI-ISOCYANATE	11.9 PPM	F	1	3	0	2	2	0	1	0	2	0 FDED					

M - male
F - female

TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX G - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

Group	Compound	Dose	Sex	24 HRS					48 HRS					72 HRS
Extended Count No. 2														
11	AIR	0 PPM	M	2	1	4	3	3						
13	TOLUENE DI-ISOCYANATE	11.8 PPM	M	7	4	5	11	6						
15	TOLUENE DI-ISOCYANATE	18.9 PPM	M	4	1	3	2	3 DEAD						
17	AIR	0 PPM	F	2	1	1	5	1	0	0	2	2	2	
19	TOLUENE DI-ISOCYANATE	7.5 PPM	F	7	4	1	0	2						
20	TOLUENE DI-ISOCYANATE	11.9 PPM	F	2	4	-	0	4	2	2	4	2	2 FDED	

M - male
F - female

TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX G - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

Group	Compound	Dose	Sex	24 HRS					48 HRS		72 HRS	
21	AIR	0 PPM	M	1	1	1	2	1				
			F	0	0	0	1	0				
22	VINYL CHLORIDE	50000 PPM	M	14	7	6	9	8				
			F	7	20	4	8	3				
23	TOLUENE DI-ISOCYANATE	5.9 PPM	M	1	5	2	0	2				
24	TOLUENE DI-ISOCYANATE	3.7 PPM	F	2	0	1	2	1				
26	TOLUENE DI-ISOCYANATE	7.5 PPM	F	2	2	2	0	1				

M - male
F - female

TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX H

INDIVIDUAL ANIMAL DATA - % POLYCHROMATIC ERYTHROCYTES

Group	Compound	Dose	Sex	24 HRS					48 HRS					72 HRS				
11	AIR	0 PPM	M	37.9	41.6	41.3	38.6	32.0	38.4	39.3	40.1	43.5	42.0	37.1	38.2	39.1	43.6	47.7
12	VINYL CHLORIDE	50000 PPM	M	37.2	41.5	41.6	39.4	23.1										
13	TOLUENE DI-ISOCYANATE	11.8 PPM	M	38.0	47.8	27.7	35.9	44.8										
15	TOLUENE DI-ISOCYANATE	18.9 PPM	M	15.0	33.3	30.2	31.6	25.3	32.8	34.8	41.3	DEAD	10.3	38.8	33.3	25.8	40.9	37.6
17	AIR	0 PPM	F	45.0	38.2	44.2	47.7	33.3	37.9	37.8	22.1	38.1	37.8	41.6	33.3	28.5	34.9	47.0
18	VINYL CHLORIDE	50000 PPM	F	41.4	25.1	38.4	38.4	29.2										
19	TOLUENE DI-ISOCYANATE	7.5 PPM	F	33.1	42.1	35.7	39.0	45.7										
20	TOLUENE DI-ISOCYANATE	11.9 PPM	F	33.0	36.7	37.9	31.7	30.9	33.0	35.0	26.4	25.0	28.0	37.7	FDED	40.6	25.7	28.8

M - male
F - female

TOLUENE DI-ISOCYANATE: AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX H - continued

INDIVIDUAL ANIMAL DATA - % POLYCHROMATIC ERYTHROCYTES

Group	Compound	Dose	Sex	24 HRS	48 HRS	72 HRS
21	AIR	0 PPM	M	49.7 50.5 46.0 51.9 44.0		
			F	47.9 49.5 49.5 42.6 47.2		
22	VINYL CHLORIDE	50000 PPM	M	33.7 49.6 44.8 48.1 49.2		
			F	51.3 38.9 49.5 42.5 40.6		
23	TOLUENE DI-ISOCYANATE	5.9 PPM	M	43.0 45.3 51.0 41.6 49.6		
24	TOLUENE DI-ISOCYANATE	3.7 PPM	F	42.7 44.7 42.8 44.5 44.7		
26	TOLUENE DI-ISOCYANATE	7.5 PPM	F	38.2 45.6 31.0 34.9 50.7		

M - male
F - female

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX I

INDIVIDUAL BODYWEIGHTS (g) - PHASE II

Animal Number	Bodyweight (g)	Animal Number	Bodyweight (g)
101	27.2	141	27.2
102	26.1	142	27.7
103	25.2	143	25.7
104	23.1	144	28.4
105	29.2	145	29.8
111	23.3	151	25.4
112	27.1	152	29.5
113	28.1	153	26.8
114	24.7	154	27.4
115	27.4	155	27.5
121	24.2	161	25.9
122	25.7	162	29.2
123	27.6	163	27.2
124	27.6	164	27.5
125	25.9	165	26.0
131	24.9	171	27.6
132	27.0	172	28.5
133	27.7	173	30.7
134	25.1	174	25.9
135	25.7	175	27.4

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX I - continued

INDIVIDUAL BODYWEIGHTS (g) - PHASE II

Animal Number	Bodyweight (g)	Animal Number	Bodyweight (g)
181	20.0	201	19.0
182	18.0	202	19.0
183	18.0	203	20.0
184	17.0	204	18.0
185	21.0	205	18.0
186	18.0	206	19.0
187	16.0	207	19.0
188	22.0	208	21.0
189	20.0	209	18.0
190	18.0	210	19.0
191	18.0	211	20.0
192	19.0	212	19.0
193	19.0	213	19.0
194	18.0	214	18.0
195	18.0	215	19.0
196	21.0	216	19.0
197	18.0	217	19.0
198	20.0	218	21.0
199	18.0	219	19.0
200	18.0	220	20.0

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX I - continued

INDIVIDUAL BODYWEIGHTS (g) - PHASE III

Animal Number	Bodyweight (g)	Animal Number	Bodyweight (g)
221	23.6	241	25.1
222	23.8	242	26.0
223	24.0	243	23.1
224	24.3	244	23.4
225	21.2	245	24.9
226	19.5	246	20.3
227	17.5	247	19.5
228	16.3	248	15.4
229	19.0	249	15.5
230	19.5	250	21.3
231	23.1	251	17.7
232	22.1	252	24.0
233	22.3	253	22.2
234	24.2	254	21.6
235	19.9	255	20.8
236	17.3	256	20.1
237	17.3	257	19.9
238	17.6	258	17.7
239	20.5	259	20.5
240	18.5	260	19.2

TOLUENE DI-ISOCYANATE:
AN EVALUATION IN THE MOUSE MICRONUCLEUS TEST

APPENDIX I - continued

INDIVIDUAL BODYWEIGHTS (g) - PHASE III

Animal Number	Bodyweight (g)	Animal Number	Bodyweight (g)
261	22.5	266	19.8
262	26.0	267	19.4
263	24.8	268	17.2
264	22.7	269	16.9
265	23.9	270	19.9

CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 8 (Month) 20 (Day) 93 (Year) Marcia Lubelius Camera Operator

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